Noncovalent Interactions of Platinum(II) Square Planar Complexes Containing Ligands Out-of-Plane with DNA

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The interaction of the complexes $[Pt(bipy)(4\text{-}Rpy)_2]^{2+}$ and $[Pt(4,4'\text{-}Ph_2bipy)(4\text{-}Rpy)_2]^{2+}$ (Ph = phenyl; bipy = 2,2′-bipyridine; R = H, CN, CH_3, NH_2) with DNA has been studied with a series of techniques. The processes give rise to (i) lengthening of rodlike DNA and unwinding of closed circular DNA and (ii) an increase in the DNA melting temperature comparable with that observed for known intercalators. In addition, the reaction of the complexes $[Pt(bipy)(py)_2]^{2+}$ and $[Pt(4,4'\text{-}Ph_2bipy)(py)_2]^{2+}$ is inhibited by the presence of DNA. These results have been interpreted by assuming that the substances intercalate in spite of the presence of ligands out of plane. The crystal structure determined for $[Pt(4,4'\text{-}Ph_2bipy)(3,5\text{-}Me_2py)_2]^{2+}$ by X-ray analysis shows that also one of the phenyl rings is twisted with respect to the square plane. Binding constants, K_B, determined spectrophotometrically at 25 °C and pH 7 using the McGhee−von Hippel approach, increase for both series of complexes on increasing pK_a of coordinated pyridines and are larger for those with 4,4′-Ph_2bipy. The increasing affinity for DNA on increasing electron density of the interacting moiety is accounted for by assuming that London dispersion forces play a major role in the processes.

Introduction

Metal complexes interact noncovalently[1] with double-helix DNA in two basic ways: external binding and intercalation. External binding includes all those interactions which occur on the DNA surface, the simplest of which is capture of the complex by the ionic atmosphere of the biopolymer. Groove binding is a much stronger type of interaction and occurs when a molecule with proper size and shape docks in one of the grooves of DNA. Intercalation consists of the insertion of a flat aromatic drug molecule between two adjacent bases at the core of the macromolecule. The steric features of octahedral and tetrahedral complexes are unsuitable for intercalation, and these substances, as a rule, bind externally.[2] On the contrary, for square planar complexes containing aromatic moieties, intercalation is the common binding mode.[3] The introduction of bulky substituents in flat aromatic molecules has been shown[4] to prevent their intercalation to DNA; it is expected, therefore, that the presence of ligands out-of-plane in square planar complexes may favor switching from intercalation to external binding. With this point in mind we have studied the interactions of the complexes $[Pt(4,4'\text{-}Ph_2bipy)(4\text{-}Rpy)_2](PF_6)_2$ (Ph = phenyl; bipy = 2,2′-bipyridine; R = CN, H, CH_3, NH_2) and $[Pt(4,4'\text{-}Ph_2bipy)(3,5\text{-}Me_2py)_2](PF_6)_2$ (Me = methyl) with calf thymus DNA. In these complexes the pyridines are markedly twisted[5] with respect to the square plane; additional deviation from planarity due to the presence of phenyl substituents occurs. The corresponding complexes with ethylenediamine instead of pyridine are known to bind by intercalation.[6] In a preliminary investigation[7] of the complexes $[Pt(bipy)(py)_2]^{2+}$ we have observed a linear relationship between the logarithm of the association constant with DNA and the pK_a of coordinated pyridine and suggested that the complexes intercalate. In this paper we report further evidence of intercalation of these complexes and show that also those with 4,4′-Ph_2bipy intercalate in spite of the additional deviation from planarity due to the presence of phenyl substituents at bipy. The molecular structure of $[Pt(4,4'\text{-}Ph_2bipy)(3,5\text{-}Me_2py)_2](PF_6)_2$ determined by X-ray analysis is also reported.

Experimental Section

Materials. (i) Complexes. The preparation of the complexes $[Pt(bipy)(4\text{-}Rpy)_2](PF_6)_2$ has already been reported.[7] The complexes $[Pt(4,4'\text{-}Ph_2bipy)(py)_2](PF_6)_2$ were prepared by reacting $[Pt(DMSO)_2Cl_2]$, dissolved in a small amount of DMSO (dimethyl sulfoxide), with 4,4′-Ph_2bipy in C_6H_6. The resulting solution was heated for about 30 min, and the yellow orange precipitate that formed was filtered off

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and washed with methanol. The substance was then suspended in water−methanol and heated until dissolution in the presence of an excess (about 100 times) of the appropriate pyridine. The final complexes $[Pt(4,4'\text{-}Ph_2bipy)(4\text{-}Rpy)_2](PF_6)_2$ were obtained as yellow precipitates by addition of NH_4PF_6 and crystallized from acetonitrile. The NMR characterization of the substances is reported elsewhere.[8] $[Pt(4,4'\text{-}Ph_2bipy)(3,5\text{-}Me_2py)_2](PF_6)_2$ was also characterized in solid by single-crystal X-ray analysis.

(ii) DNA. Calf thymus DNA was purchased from Sigma Chemical Co. and purified as previously described.[6d] DNA concentration, expressed in base pairs, was calculated spectrophotometrically using an $\epsilon_{260\ nm}$ value of $1.31 \times 10^4\ M^{-1}\ cm^{-1}$.[9] pBR322 plasmid DNA, isolated from *Escherichia coli*, ATCC 37017, was purchased from Sigma Chemical Co. and used as received.

DMSO, NaCl, and other chemicals were of reagent grade and were used without further purification.

Methods. All experiments were carried out at 25 °C, pH 7, in a phosphate buffer and enough NaCl to give the desired ionic strength.

The pH was measured with a Radiometer PHM 62.

Absorption spectra were recorded using a Lambda 5 Perkin-Elmer spectrophotometer.

NMR. ^1H and $^{13}C\{^1H\}$ spectra were recorded on a Bruker ARX-300 spectrometer at 300.13 and 75.44 MHz, respectively.

Thermal Denaturation Experiments. The thermal denaturation temperature of complex−DNA mixtures (1:10) was determined in 1×10^{-3} M phosphate buffer (pH 7) containing 7.8×10^{-6} M complex and 2×10^{-3} M NaCl. Melting curves were recorded at 260 nm on a Lambda 3-B Perkin-Elmer spectrophotometer interfaced with a 486 IBM computer. The temperature was increased at a rate of 0.5 °C/min by using a PTP-1 Peltier Perkin-Elmer temperature programmer.

Viscometry. A Cannon-Ubbelhode semi-microdilution viscometer (series no. 75, Cannon Instruments Co.), thermostatically maintained at 25 °C in a water bath, was used for viscosity measurements. The viscometer contained 2 mL of sonicated DNA solution. The complex solution ($(1-2) \times 10^{-4}$ M), containing also DNA (4×10^{-4} M) at the same concentration as that in the viscometer, was delivered in increments of 50−80 μL from a micropipet. Solutions were freed of particulate material by passing them through Acrodisc CR PTFE syringe filters before use. Flow times were measured with a digital stopwatch. Reduced viscosities were calculated by established methods and plotted as $\ln \eta/\eta^\circ$ against $\ln(1 + r)$ for rodlike DNA (600 base pairs) (η is the reduced viscosity of the DNA solution in the presence of complex; η°, the reduced viscosity of the DNA solution in the absence of complex; and r $[complex]_{bound}/[DNA]_{tot}$) and as η/η° against r for covalently closed DNA.

Binding Constant Determination. Spectrophotometric titrations were performed by adding to a complex solution $[(5.0-8.5) \times 10^{-5}$ M] successive aliquots of DNA, containing also the complex, in a 10 mm stoppered quartz cell and recording the spectrum after each addition. The data were analyzed by a non-least-squares fitting program, applied to the McGhee and von Hippel equation.[10] The binding constant K_B and the cooperativity parameter ω were determined by the program, using the extinction coefficient, free complex concentration, and the ratio of bound complex per mole of DNA. The computer program varied the cooperativity parameter ω. Extinction coefficients for bound compounds were determined by Beer's law plots in the presence of a large excess of DNA.

Kinetics. The rates of reaction were followed spectrophotometrically using a Perkin-Elmer Lambda 5 spectrophotometer. In all cases a large excess of nucleophile was used to provide pseudo-first-order conditions and to force the reaction to completion. Pseudo-first-order rate constants k_{obsd} were obtained from nonlinear least-squares fits of the experimental data to $A_t = A_\infty + (A_o - A_\infty) \exp(-k_{obsd}t)$, where A_o, A_∞, and k_{obsd} were the parameters to be optimized (A_o = absorbance after mixing of the reagents, A_∞ = absorbance at completion of reaction). The k_{obsd} values were reproducible to better than ±5%.

Table 1. Details of Crystal Structure Determination for Compound $[Pt(4,4'\text{-}Ph_2bipy)(3,5\text{-}Me_2py)_2](PF_6)_2$

empirical formula	$C_{36}H_{34}F_{12}N_4P_2Pt$
fw	1007.7
color, cryst form	yellow, prismatic
cryst syst	triclinic
space group	$P\bar{1}$ (No. 2)
a, Å	10.724(2) Å
b, Å	12.850(3)
c, Å	14.878(3)
α, deg	104.46(2)
β, deg	95.36(2)
γ, deg	93.33(2)
V, Å^3	1969.6(7)
Z	2
F(000)	988
ρ(calcd), g/cm^3	1.699
$\mu[\lambda(\text{Mo K}\alpha) = 0.710\ 73$ Å], cm^{-1}	37.32
transm coeffs	0.720−0.952
T, °C	25
2θ range, deg	3−50
no. of data collcd/unique ($2\theta-\omega$)	7398/6991 ($R_{int} = 2.66\%$)
no. of data obsd	2945 [$F \geq 5\ \sigma(F)$]
no. of variables	496
R^a (obsd/all)	0.0493/0.1069
$R_w{}^a$ (obsd/all)	0.0613/0.0873
GOF^a (obsd)	0.7605

[a] Residuals calculated with $w = [\sigma^2(F) + 0.0037F^2]^{-1}$; $R = \sum||F_o| - |F_c||/\sum|F_o|$; $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$; quality of fit = GOF $= [\sum w(|F_o| - |F_c|)^2/(N_{obs} - N_{param})]^{1/2}$.

X-ray Data Collection and Structure Determination. Suitable yellow crystals of the compound were obtained by slow evaporation of 1:2 (volume) methanol−acetone solution. Diffraction data were collected at room temperature on a Siemens R3m/V automatic four-circle diffractometer using graphite-monochromated Mo Kα radiation. Lattice parameters were obtained from least-squares refinement of the setting angles of 21 reflections with $14 \leq 2\theta \leq 28°$. Table 1 lists a summary of the crystallographic data and the structure refinement.

No sign of crystal deterioration was revealed by monitoring three standard reflections after every 197 measurements. The intensities of the 7398 reflections, collected up to $2\theta = 50°$ by the variable-speed $\omega-2\theta$ scan method, were evaluated by a profile fitting among 2θ shell procedure[11] and then corrected for Lorentz−polarization effects. An absorption correction was applied by fitting a pseudoellipsoid to the azimuthal scan data of 10 high-χ reflections.[12]

The structure was solved by standard Patterson methods and subsequently completed by a combination of least-squares techniques and Fourier syntheses with the SHELXTL-PLUS system[13] (used to perform data reduction also). Whereas several hydrogens were located on final ΔF map, the H atoms were included in the refinement among the "riding model" method with the X−H bond geometry depending on the parent atom X and with an unique common fixed isotropic displacement parameters (0.085 Å^2). The refinement, with all non-hydrogen atoms anisotropic and minimizing the function $\sum w(|F_o| - |F_c|)^2$, was carried out by the full-matrix least-squares technique based on F of 2945 reflections having $F_o \geq 5\sigma(F_o)$. The model converged to $R = 0.0493$ and $R_w = 0.0613$ with the final weighting scheme $w = [\sigma^2(F_o) + 0.0037(F_o)^2]^{-1}$. In the last ΔF map the most significant electron density residuals (up to 1.22 e Å^{-3}) were located at about 1.1 Å from the Pt atom. Neutral-atom scattering factors and anomalous dispersion corrections, automatically used by the program, come from ref 14.

Final geometrical calculations and drawings were carried out with the PARST program[15] and the XP utility of the Siemens package,[13] respectively, running on a MicroVax/3400 computer. All the searches

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Table 2. Selected Distances (Å) and Angles (deg) for $[Pt(4,4'\text{-}Ph_2bipy)(3,5\text{-}Me_2py)_2](PF_6)_2$

Pt−N(1)	1.99(1)	Pt−N(2)	1.99(1)
Pt−N(3)	2.01(2)	Pt−N(4)	2.02(1)
N(1)−C(1)	1.33(2)	N(1)−C(5)	1.35(2)
C(3)−C(11)	1.48(2)	C(5)−C(6)	1.48(2)
C(6)−N(2)	1.33(2)	C(8)−C(17)	1.47(2)
C(10)−N(2)	1.37(3)	N(3)−C(23)	1.32(2)
N(3)−C(27)	1.31(3)	N(4)−C(30)	1.34(2)
N(4)−C(34)	1.33(2)		
Pt···F(6)	3.40(1)	Pt···F(10)	3.58(3)
N(1)−Pt−N(2)	79.8(5)	N(1)−Pt−N(3)	177.4(6)
N(2)−Pt−N(3)	97.7(6)	N(1)−Pt−N(4)	93.7(5)
N(2)−Pt−N(4)	173.2(5)	N(3)−Pt−N(4)	88.8(6)
Pt−N(1)−C(1)	127(1)	Pt−N(1)−C(5)	116(1)
C(1)−N(1)−C(5)	117(1)	N(1)−C(5)−C(6)	114(1)
C(5)−C(6)−N(2)	113(1)	Pt−N(2)−C(6)	117(1)
Pt−N(2)−C(10)	124(1)	C(6)−N(2)−C(10)	119(1)
Pt−N(3)−C(23)	120(1)	Pt−N(3)−C(27)	123(1)
C(23)−N(3)−C(27)	117(2)	Pt−N(4)−C(30)	121(1)
Pt−N(4)−C(34)	121(1)	C(30)−N(4)−C(34)	118(2)
Pt···F(6)−P(1)	157(1)	Pt···F(10)−P(1)	139(1)
F(6)···Pt···F(10)	173(1)		
N(4)−Pt−N(3)−C(23)	91(1)	N(3)−Pt−N(4)−C(34)	91(1)
C(4)−C(3)−C(11)−C(16)	30(2)	C(7)−C(8)−C(17)−C(18)	8(2)
N(1)−C(5)−C(6)−N(2)	2(2)		

and comparisons with the known structures have been performed within the Crystallographic Structure Database (CSD).[16] Selected geometry data of the molecule are listed in Table 2.

Results

All the complexes are very stable in pure water as well as in the presence of phosphate buffer and sodium chloride; in addition, the linear dependence of absorbance on concentration, in the range used for the experiments (2×10^{-6} to 2×10^{-4} M), rules out self-association of the complexes.

One of the most common and at the same time most sensitive ways to analyze drug−DNA interactions is UV−vis absorption spectroscopy. Figure 1 illustrates a typical spectrophotometric titration of one the 4,4′-diphenylbipyridine complexes with DNA. There is a progressive variation of the spectrum of the complex on increasing DNA amounts until a limiting spectrum is obtained. The spectral variations occur within the mixing time of the reagents, and no further reaction occurs. The inertness of the platinum(II) substrates excludes that the spectral changes are due to covalent binding of the metal to the nucleophilic sites of the bases. The noncovalent character of the process is confirmed by the total reversibility of the reaction which can be totally shifted to the left by addition of sodium chloride. Any type of noncovalent binding to DNA is associated to spectral variations; however, large hypochromism and concomitant bathochromic shifts are normally observed for intercalation.

Support to intercalation comes from thermal denaturation measurements. As well established, stacking of intercalators into the nucleobases stabilizes the double helix, increasing the melting point of DNA. The increase in thermal denaturation temperature is compared in Table 3 with that relative to the known intercalator $[Pt(bipy)(en)]^{2+}$ (en = ethylenediamine), determined under the same experimental conditions. The data clearly show that the duplex stabilization produced by the

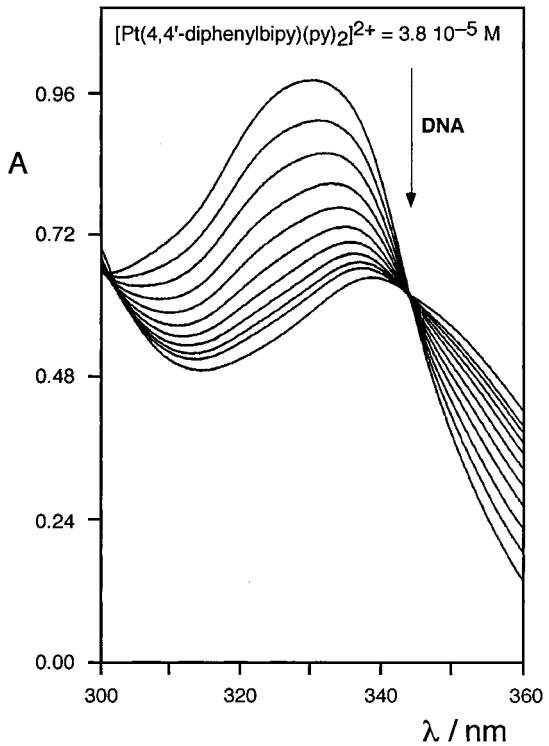

Figure 1. Spectrophotometric titration of $[Pt(4,4\text{-}Ph_2bipy)(py)_2]^{2+}$ (3.8×10^{-5} M) with DNA at 25 °C, in the presence of 4% v/v DMSO, 2.0×10^{-3} M phosphate buffer, 2.0×10^{-2} M NaCl.

Table 3. Increase in Melting Temperature of ct. DNA upon Interaction with the Complexes

complex	ΔT, °C
$[Pt(4,4'\text{-}Ph_2bipy)(py)_2]^{2+}$	9.5 (±0.1)
$[Pt(4,4'\text{-}Ph_2bipy)(3,5\text{-}Me_2py)_2]^{2+}$	10.1 (±1.2)
$[Pt(bipy)(py)_2]^{2+}$ [a]	10.4 (±0.2)
$[Pt(bipy)(4\text{-}NH_2py)_2]^{2+}$	10.3 (±0.7)
$[Pt(bipy)(en)]^{2+}$ [a]	10.4 (±0.9)

[a] Values from ref 7.

interaction with all the complexes investigated here is comparable or larger than that observed for $[Pt(bipy)(en)]^{2+}$.

A useful technique to prove intercalation is viscometry.[17] In order to accomodate the intercalator DNA must partly unwind. This produces lenghthening and contemporary stiffening in DNA which is reflected in an increase in viscosity. Figure 2 compares the increase in viscosity of rodlike DNA (600 base pairs) obtained by titration with $[Pt(4,4\text{-}Ph_2bipy)(py)_2]^{2+}$ and $[Pt(bipy)(en)]^{2+}$. The comparison shows that, under the same experimental conditions, the solution viscosity changes in the same way both with our complexes and $[Pt(bipy)(en)]^{2+}$. We have also analyzed the interaction of $[Pt(4,4\text{-}Ph_2bipy)(py)_2]^{2+}$ with closed circular supercoiled DNA. The compound (Figure 3) causes an increase, a maximum, and a subsequent decrease in a viscometric titration expected for an intercalator[18] as it first removes the DNA supercoils to an open circle and then causes reverse supercoiling of the DNA.

Finally in order to obtain further informations on the binding mode of the complexes we have followed a kinetic approach.[19] The sites of attack of an intercalated substance are sterically protected, and consequently its reactivity largely decreases. On

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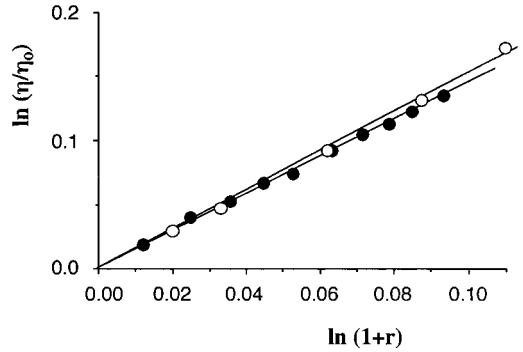

Figure 2. Viscometric titration with ct. DNA of (○) $[Pt(bipy)(en)_2]^{2+}$ and (●) $[Pt(4,4\text{-}Ph_2bipy)(py)_2]^{2+}$, in 1.0×10^{-3} M phosphate buffer (pH 7) and 1.0×10^{-2} M NaCl. η is the intrinsic viscosity of sonicated DNA in the presence of the complex; η_o, the intrinsic viscosity of sonicated DNA in the absence of the complex; r, the number of drug molecules bound to base pairs.

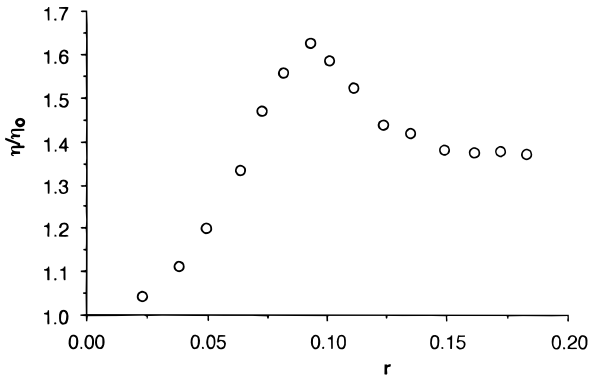

Figure 3. Viscometric titration with ccs DNA of $[Pt(4,4\text{-}Ph_2bipy)(py)_2]^{2+}$, in 1.0×10^{-3} M phosphate buffer (pH 7) and 1.0×10^{-2} M NaCl. r is the number of drug molecules bound to base pairs.

the contrary, when the substance is only externally bound, and thanks to its positive charge concentrates on DNA environment, its reactivity with respect to neutral or cationic reagents increases. The effect of DNA on the reactivity of a substance can, therefore, be related to its binding mode with the biopolymer. We have followed kinetically the reaction of replacement of the first pyridine, by thiourea (tu), in the complexes $[Pt(4,4'\text{-}R_2bipy)(py)_2]^{2+}$ (R = H, C_6H_5). Thiourea is an ideal reagent for this type of study; it is a very effective nucleophile toward square planar complexes and being neutral is not repelled by DNA surface. The reaction is associated to large spectral variations in the UV−visible region and, therefore, it has been monitored spectrophotometrically.

$$[Pt(4,4'\text{-}R_2bipy)(py)_2]^{2+} + tu \rightarrow [Pt(4,4'\text{-}R_2bipy)(py)(tu)]^{2+} + py \quad (1)$$

Under pseudo-first-order conditions with respect to the complex, the observed rate constant k_{obsd} is linearly correlated (Figure 4) to thiourea concentration:

$$k_{obsd} = k_2[tu] \quad (2)$$

At 25 °C the k_2 values are 0.105 and 0.104 M^{-1} s^{-1}, respectively, for $[Pt(bipy)(py)_2]^{2+}$ and $[Pt(4,4'\text{-}Ph_2bipy)(py)_2]^{2+}$. In the presence of DNA the rates of both reactions decrease, and when the amount of added DNA is large enough to force the interaction to completion, the reactions appear to be totally inhibited. Such a kinetic effect is in line with an intercalative mode of binding of the substances to DNA.

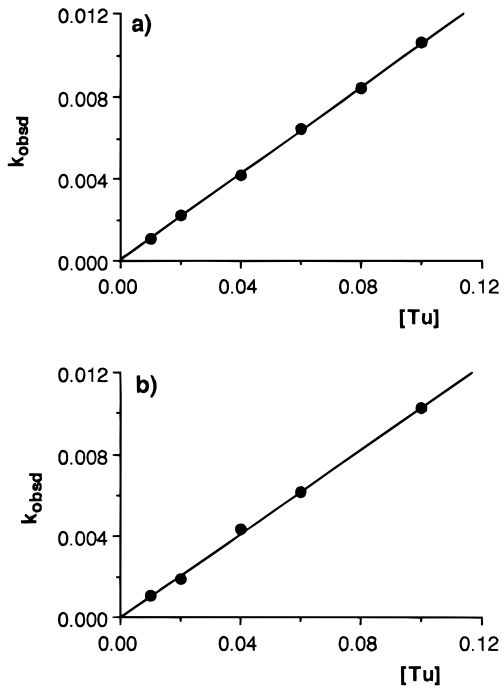

Figure 4. Plot of k_{obsd} (s^{-1}) vs [tu] for reaction (1): (a) $[Pt(bipy)(py)_2]^{2+}$; (b) $[Pt(4,4\text{-}Ph_2bipy)(py)_2]^{2+}$.

In conclusion, although in the absence of X-ray structure determinations groove binding can never be excluded, the results of all experiments are concordant and strongly indicate that the presence in the complexes of groups out-of-plane does not prevent intercalation.

The results of the spectrophotometric titrations of the complexes with DNA have been reported in the form of Scatchard[20] plots of r/m vs m, where r is the binding ratio $[complex]_{bound}/[DNA]_{tot}$ and m is the concentration of unbound complex. The data were analyzed via the McGhee−von Hippel[10] equation based on the nearest-neighbor exclusion model using a nonlinear least-squares computer program.[7]

Binding values were too high to be determined without uncertainty at 0.022 M ionic strength; therefore the spectrophotometric titrations were run at 0.22 M ionic strength value, taking advantage of the destabilizing effect[21] of this parameter on the interactions with DNA. In addition, owing to the reduced solubility in water of the complexes with 4,4′-Ph_2bipy, the determinations were conducted in the presence of 4% v/v dimethyl sulfoxide; this substance lowers the K_B values (for the complex $[Pt(4,4'\text{-}Ph_2bipy)(py)_2]^{2+}$, the only complex soluble enough to obtain binding constants in water, the K_B values at 25 °C and 0.22 M ionic strength are 3.64×10^5, 2.58×10^5, 2.81×10^5, and 1.67×10^5, respectively, in the presence of 0, 0.4, 1.4, and 4% v/v DMSO) but does not alter the trend of the processes as shown by the substantial identity of the spectral variations on titrating the complexes with DNA in the absence and in the presence of DMSO. Approximate values of the binding constants at 0.022 M ionic strength were estimated by assuming, in accordance with the polyelectrolyte theory,[21] that for a dicationic intercalator, when the sodium concentration is increased, by a factor of 10, the K_B value decreases by about 100 times. These values are collected in Table 4.

The number of excluded sites ranges between 1 and 2 and the ω value, in all cases very close to unity, shows a lack of

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Table 4. Values of Binding Constants (K_B, M^{-1}) for the Interaction of the Complexes $[Pt(bipy)(4\text{-}Rpy)_2]^{2+}$ and $[Pt(4,4'\text{-}Ph_2bipy)(4\text{-}Rpy)_2]^{2+}$ with Calf Thymus DNA, in 2.0×10^{-3} M Phosphate Buffer (pH = 7), $T = 25$ °C, 2.0×10^{-2} M NaCl

complex	$10^{-4}K_B$,[a] M^{-1}	complex	$10^{-6}K_B$,[b] M^{-1}
$[Pt(bipy)(4\text{-}CNpy)_2]^{2+}$	1.5 (±0.1)	$[Pt(4,4'\text{-}Ph_2bipy)(4\text{-}CNpy)_2]^{2+}$	4.0
$[Pt(bipy)(4\text{-}Ph\text{-}py)_2]^{2+}$	13.2 (±1.2)		
$[Pt(bipy)(py)_2]^{2+}$	16.2 (±0.2)	$[Pt(4,4'\text{-}Ph_2bipy)(py)_2]^{2+}$	16.7
$[Pt(bipy)(4\text{-}Me\text{-}py)_2]^{2+}$	27.0 (±0.7)	$[Pt(4,4'\text{-}Ph_2bipy)(4\text{-}Me\text{-}py)_2]^{2+}$	28.9
$[Pt(bipy)(4\text{-}NH_2py)_2]^{2+}$	116.7 (±9.0)	$[Pt(4,4'\text{-}Ph_2bipy)(4\text{-}NH_2py)_2]^{2+}$	140.0

[a] Values from ref 7. [b] Extrapolated values; in the presence of 4% v/v DMSO.

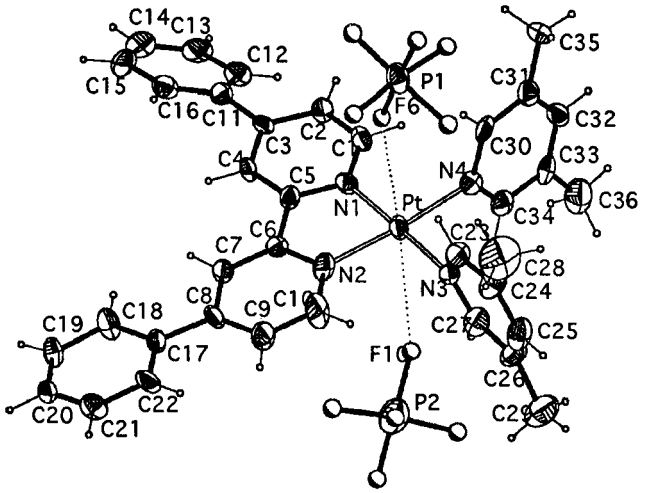

Figure 5. View of the asymmetric unit showing the $[Pt(4,4'\text{-}Ph_2bipy)(3,5\text{-}Me_2py)_2](PF_6)_2$ aggregate with the labeling scheme. The axial Pt−F long interactions are represented by dotted lines. Thermal ellipsoids are drawn at 30% of probability while hydrogen and hexafluorophosphate atom sizes are arbitrary.

cooperative interactions between the complexes intercalated in neighboring positions.

Crystal Structure of $[Pt(4,4'\text{-}Ph_2bipy)(3,5\text{-}Me_2py)_2](PF_6)_2$. The crystallographic asymmetric unit, represented in Figure 5, contains one dicationic complex of platinum(II) that interacts with both the hexafluorophosphate anions, constituting one neutral discrete aggregate in the solid state. The Pt^{2+} atom shows the usual square planar geometry but on each axial position is placed one fluorine of a $[PF_6]^-$ at a distance 3.40(1) and 3.58(3) Å, respectively.

The geometry of the complex is mainly determined by the flat 4,4′-Ph_2bipy ligand chelating the platinum(II) on the same coordination plane: with respect to the weighted least-squares plane passing through bipyridine and PtN_4 fragments the atom deviation ranges from −0.04(1) Å for C(4) to 0.05(1) Å for N(4). Even though the four Pt−N bond lengths are equivalent within the experimental errors, the square planar geometry of the Pt atom is deformed by the short coordination bite of the 4,4′-diphenylbipy ligand which causes the contraction on the N(1)−Pt−N(2) angle up to 79.8(5)° that falls into the range 74−84° observed for the known bipy−Pt chelates. The two phenyl substitutents of 4,4′-Ph_2bipy are rotated 31.3(6)° from each other to minimize possible hindering effects, and with respect to the ligand mean plane the C(11−16) ring forms a dihedral angle of 32.6(4)° while that for C(17−22) is only 6.9(3)°. This rotation difference, not influencing the corresponding $C_{Ph}-C_{bpy}$ bond lengths, might be due to the crystal packing as evidenced by significant different intermolecular phenyl···$[PF_6]^-$ contacts that are related to the different distance of the Pt-F long interactions also.

The two 3,5-dimethylpyridine ligands are disposed orthogonally to the mean coordination plane forming the dihedral angles 90.6(4) and 87.0(3)° to minimize the significant steric hindrance between their *m*-methyl substitutents. This arrangement of the Pt complex is similar to the reported geometry of the comparable (4,4′-dimethyl-2,2′-bipyridyl)bis(3,5-dimethylpyrazole)platinum-(II).[22]

NMR experiments on $[Pt(4,4'\text{-}Ph_2bipy)(4\text{-}CNpy)_2](PF_6)_2$ confirm that the structure of these type of complexes is mantained in solution. 2D inverse heterocorrelated NMR spectra and NOE were used to assign the proton and carbon resonances. The ^1H and $^{13}C\{^1H\}$ spectra in acetone-d_6 at 210 K do not show significant broadening. This indicates that both pyridines and phenyl rings freely rotate also at this temperature. The C_o spin−lattice relaxation time constants provide evidence of fast anisotropic motion of the phenyl rings around the C_c-C_f axis (relaxation time constants of C_g and C_h are about twice as long as that of C_i). This implies that there are no important restrictions to the formation of large aromatic coplanar π systems.

Discussion

The classical model of intercalation[23] implies insertion of the whole molecule between the base pairs. Simple considerations on the molecular structure of the complexes studied show that this model cannot be applied here. As is known[5] two pyridines bound to the central metal in relative cis position do not lie in plane and must assume a skew position; as a consequence this part of the complex is too large to intercalate. The substances can, however, slip between two adjacent base pairs through bipyridine intercalating partially with the pyridines confined out of the nucleobases. The behavior of these substrates recalls partial intercalation proposed[24] for some trischelate octahedral complexes where only one of the ligands intercalates. Obviously for the latter substances the requirements for the process are much more severe due to the steric interference of two nonintercalated ligands with the surrounding DNA structure.

Our experimental findings show that additional deviation from planarity, on the side of bipyridine, due to the presence of a phenyl group out of plane, does not prevent intercalation; on the contrary on going from the bipyridine complexes to the 4,4′-diphenylbipyridine complexes the binding avidity for DNA largely increases (see later in the Discussion). Intercalation of these complexes implies that the phenyl groups rotate into the plane of the bipyridine moiety so maximizing stacking. Perfect coplanarity of the four rings is, however, unnecessary; it has been shown[25] that some twist about the torsional bonds joining the aromatic rings may complement or enhance the intrinsic

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propeller twist of DNA base pairs. If the rotational barrier makes the process unfavored it could be proposed, alternatively, that the base pairs open up[26] during the process of intercalation to accommodate the skewed phenyl group.

All the complexes show strong binding affinity (Table 4) toward DNA and the K_B values relative to the complexes with 4,4′-Ph_2bipy, are larger. The higher binding affinity of the latter complexes is to be attributed to the larger stacking surface of $[Pt(4,4\text{-}Ph_2bipy)(4\text{-}Rpy)_2]^{2+}$ due to the presence of two additional phenyl rings. Data of Table 4 show also that $[Pt(4,4\text{-}Ph_2bipy)(py)_2]^{2+}$ is a much more potent intercalator than $[Pt(bipy)(4\text{-}phenylpy)_2]^{2+}$; this is evidence that the two phenyl groups of 4,4′-Ph_2bipy must be, at least partially, intercalated. Furthermore, within each set of complexes there is also a strong dependence of the binding affinity on the nature of the substituent at pyridine.

The effect of groups bound to intercalators on the association with DNA has been evaluated quantitatively for many systems;[27,28] contrasting results have emerged, however, in comparing intercalators differing by the nature of the substituents. For instance, the presence of groups markedly different by electron donor properties such as CN and OCH_3 at the 6-position in *N*-(3-(dimethylamino)propyl)naphtho[2,1-*b*]thiophene-4-carboxamide leads in both cases to increase in binding[27] affinity for DNA with respect to the unsubstituted substance. The reason for such a result is that the introduction of a substituent directly at the intercalating moiety alters the chemical and/or the steric properties of the intercalator giving rise to effects specific of the substituents and often dominant in the interaction process. Our complexes are free from these limitations: the substituents are located in a remote position with respect to the moiety directly involved in the interaction; steric and chemical integrity of the intercalating unit is preserved along the series of substances. The data show that K_B values increase systematically on increasing pK_a of the substituted pyridine. As increasing pK_a means increasing σ donor power of pyridine nitrogen, it follows that bipyridine charge density is enhanced when pyridine pK_a increases. Thus a direct dependence of binding affinity on electron density of the intercalating moiety does exist. The values of logarithms of K_B for both series of substances are linearly correlated to the pK_a values of coordinated pyridines (Figure 6) with the 4,4′-Ph_2bipy complexes showing much greater binding affinity for DNA.

The increase of binding affinity on increasing electron density of the intercalating unit is remarkable; in previous reports, in fact, it has been pointed out that the stacking between nucleobases and aromatic rings is disfavored by electron-rich centers. For instance, Marzilli et al.,[29] in comparing the interactions with DNA of two cationic porphyrins different significantly by electronic properties, concluded that intercalation requires an electron-deficient core. The enhancement of binding affinity

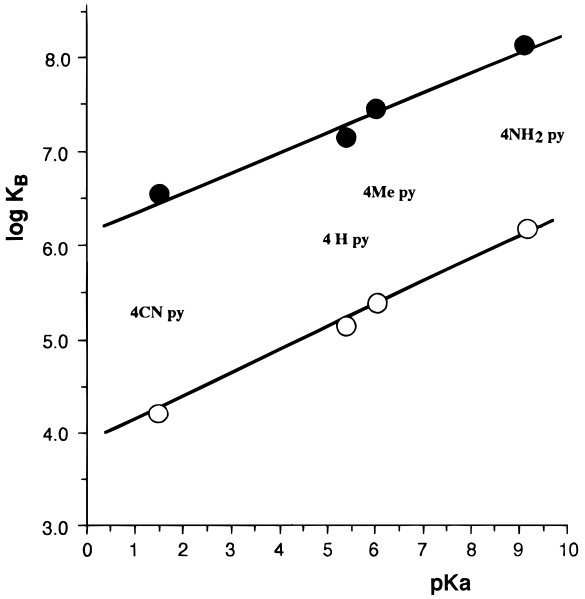

Figure 6. Plot of log K_B vs pK_a of coordinated pyridines for the complexes (○) $[Pt(bipy)(4\text{-}Rpy)_2]^{2+}$* and (●) $[Pt(4,4'\text{-}Ph_2bipy)(4\text{-}Rpy)_2]^{2+}$. (*Values from ref 7.)

on increasing electron density of the intercalating moiety of a drug underlines the importance of London dispersion forces[30] in these processes. Also charge transfer interactions can play important roles in aromatic−aromatic interactions, but in the present case, these interactions do not seem significant. Yamauchi et al.[31] in a series of related papers have studied the adduct formation between cationic platinum(II) complexes of the type $[Pt(L)(en)]^{2+}$ (L = variously substituted 2,2′-bipyridine and 1,10-phenanthroline) and a series of mononucleotides and have shown that the electron donor amino group of adenosine 5′-monophosphate and guanosine 5′-monophosphate enhance the charge transfer to phenanthroline, increasing the stability of the adduct with $[Pt(phen)(en)]^{2+}$ with respect to inosine 5′-monophosphate where the amino group is absent. Accordingly, increasing density charge of coordinated phenanthroline should reduce charge transfer from the nucleobase and the resulting stability of the adducts. The observed K_B trend, however, is opposite, suggesting that, in the processes investigated here, van der Waals interactions are more important than CT interactions.

In conclusion, our complexes constitute a new class of partial intercalators with exceptionally high binding affinity for DNA, and London dispersion forces play a major role in the interaction with DNA.

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Supporting Information Available: X-ray crystallographic files, in CIF format, are available on the Internet only. Access information is given on any current masthead page.

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